学位論文

Doctor's Thesis

Magnetic resonance imaging of autologous chondrocyte implantation in the knee joint

（自家軟骨培養移植術を施行した膝関節に対する磁気共鳴画像に関する研究）

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Magnetic Resonance Imaging of Autologous Chondrocyte Implantation in the Knee Joint

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CONTENTS

SUMMARY

Purpose

The purpose of this study is to evaluate appearance of autologous chondrocyte implantation (ACI) and overall state of the knee based on magnetic resonance imaging (MRI) at one year postoperatively. and to assess whether other knee pathologies or features affect the graft quality and to compare these findings with clinical outcome at one postoperative year. This study also was performed to compare the MRI appearances of ACI grafts with graft histology.

Material and Methods

Forty-nine patients (35 men. 14 women; age range. 18-51; mean age 35.4 years) underwent ACI procedure of a femoral condyle in the knee. MRI was performed preoperatively and at one year postoperatively. and graft biopsy was performed at one year postoperatively.

Standard T1-, T2-, T2*-. and intermediate-weighted sequences were performed. as well as three-dimensional (3D) fast low-angle shot (FLASH) and double-echo signal-state (DESS) sequences for cartilage assessments. The ACI grafts were assessed for surface smoothness. thickness. in comparison with that of adjacent cartilage. signal intensity. integration to underlying bone and adjacent cartilage. contgruity of subchondral bone. Presence of overgrowth and bone marrow appearance were also assessed. Presence of osteophyte formation. further cartilage defects. appearance of

cruciate ligaments and menisci in the knee were also recorded. An overall grafts score was constructed using the graft appearances. This was correlated with the knee features and the Lysholm score, a clinical self-assessment score. Graft biopsy results were categorized into those showing hyaline, mixed fibrohyaline cartilage, fibrocartilage, and fibrous tissue. The ACI graft features were correlated with the histological findings.

Results

Of 49 grafts, 32 (65%) demonstrated complete filling 1 year postoperatively. General overgrowth was seen in eight grafts (16%), and partial overgrowth in 13 grafts (26%). Bone marrow change underneath the graft was seen; oedema was seen in 23 grafts (47%), cysts in six grafts (12%) and sclerosis in two grafts (4%). Mean graft score was 8.7 (of maximal 12) (95% CI; 8.0-9.5). Knee without osteophyte formation or additional other cartilage defect (other than the graft site) had a significantly higher graft score than knees with multiple osteophytes (P=0.0057) or multiple further cartilage defects (P=0.014). At 1 year follow-up, improvement in the clinical scores was not significantly different for any subgroup. Knee with a graft score of 8 points or greater had a better improvement of the clinical score than those of 7 points or fewer.

Histological assessment of the graft was performed in 41 grafts. The graft consisted of hyaline cartilage in four (10%), mixed fibrohyaline cartilage in 10 (24%), fibrocartilage in 25 (61%), fibrous tissue in two (5%) cases. Graft intensity was virtually always lower than adjacent normal cartilage signal intensity, and there was no

relationship between graft signal intensity and histological appearance (P=0.34). Graft thickness (P=0.83), overgrowth (P=0.69), surface smoothness (P=0.28), and integration with adjacent cartilage and underlying bone (P=0.90), edema in bone marrow underneath graft (P=0.63), and bone contour underneath graft (P=0.94) at MRI had no correlation with graft histological appearance.

Conclusions

ACI grafts on MRI at one year follow up have a spectrum of appearances. Bone marrow oedema deep to the graft and graft overgrowth are common and do not appear to have an adverse prognostic significance. Grafts with advantageous features such as good integration to adjacent cartilage and underlying bone and no significant thickness defects are more frequently found in otherwise well preserved knees. The presence of osteophytes and other cartilage defects results in poorer ACI grafts at 1 year. Advantageous graft and knee features are associated with a better absolute clinical outcome but not with the relative improvement. In other words, better grafts are associated with clinically better knees, but, even for suboptimal grafts, there is improvement. However, MRI cannot predict ACI graft histology; histology was not related to graft signal intensity, thickness, overgrowth, surface smoothness, integration to adjacent cartilage or underlying bone, and signal change in underlying bone marrow.

MRI of ACI grafts allows for serial follow up of patients non-invasively. It can assess the entire graft and its integration to adjacent bone and cartilage. It allows

assessment of the entire remainder of the knee. With development of higher field scanners and progress in sequence development it can be expected that resolution and contrast will increase while imaging time decreases.

PUBLICATION LIST

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2. Tins BJ, McCall IW, Takahashi T, Cassar-Pullicino V, Roberts S, Richardson JB, Ashton B. Autologous chondrocyte implantation in knee joint: MR imaging and histologic features at 1-year follow-up. *Radiology*, 2005 Feb;234(2):501-8. Epub 2004 Dec 22.

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ABBREVIATIONS

ACI: autologous chondrocyte implantation

MR: magnetic resonance

MRI; magnetic resonance imaging

3-D; three dimensional

FLASH; fat-suppressed fast low-angle shot

DESS; double-echo steady state

INTRODUCTION

Articular cartilage has an important role in decreasing the impact of a mechanical load and in decreasing friction in normal joints. The degeneration of articular cartilage and alternations in other joint tissue result from the loss of structure and function of damaged cartilage. Although articular cartilage is metabolically active, adult articular cartilage has a limited capacity to repair structural damage resulting from injury. Because of this limitation, the damaged joint often loses the articular surface exposing the underlying the bone. These changes cause a severely impaired joint function which is called as osteoarthritis, and almost always lead to loss of motion, swelling, redness and pain. Artificial joint replacements offer the best hope restoring pain-free joint to patients suffering from painful joint. Although joint replacements have brought revolution to surgeons, the problem of the durability and limited range of motion has not been solved.

Therefore, biological replacement would be ideal procedure to restore joint function. Surgeons have developed several procedures intended to improve articular cartilage repair, and thereby improve joint function, decrease pain, and prolong the progression of osteoarhtritis. The most common of these procedures can be grouped into three categories as 1) local stimulation for cartilage repair, 2) autologous transplantation of cartilage and 3) allograft transplantation.

Local stimulation for cartilage repair

The three operative procedures for local stimulation for cartilage repair of full-thickness lesions are abrasion arthroplasty, microfracture and subchondral drilling. All local stimulation techniques rely on formation of a fibrin clot within the defect that is created by bleeding from the penetration of the subchondral bone underlying the chondral lesion. The fibrin clot contains pluripotent stem cells that differentiate and remodel, leading to the formation of repair tissue. The repair tissue has been reported to be fibrocartilage or a hybrid of fibrocartilage and hyaline cartilage.

Local stimulation techniques differ in the manner in which the subchondral bone is violated. In abrasion arthroplasty, a burr is used to penetrate the bone, whereas in subchonral drilling, a drill is used to perform this function. With microfracture, a pick or awl is used to make multiple penetrations into the subchondral bone. Local stimulation techniques are generally recommended for lesions smaller than 4 cm^2.

Autologous transplantation of cartilage

Some patients have medium or large full-thickness articular cartilage lesions of the knee, which are too large or have poor borders to consider microfracture, subchondral drilling, or abrasion arthroplasty. For these patients, two surgical procedures, which have gained popularity to address this, are autologous osteochondral transplantation (AOT) and autologous chondrocyte implantation (ACI). Both have attracted a great deal of interest because of their potential to form hyaline or hyaline-like

repair tissue.

Autologous Osteochondral Transplantation (AOT)

Autologous osteochondral transplantation is also known as mosaicplasty. AOT involeves the use of cylinderic osteochondral plugs of various sizes to fill chondral defects. The plugs are harvested from less important weight bearing articular surfaces. These harvest sites are usually the borders of the trochlea and interchondylar notch area of the knee. The chondral defect being repaired is debrided down to viable subchondral bone, and the plugs are transplanted into the defect site.

Theoretical advantages of osteochodral transplantation are that they provide islands of hyaline cartilage with fibrocartilage "grout", can be done in one procedure, maintain a normal cartilage bone interface, rely of bone to bone healing of the grafts, and can add bone to an osteochondral lesion such as, osteoarthritis dissecans. Disadvantages include the fact that the harvest sites though not as important weight bearing articular surfaces are still articular surfaces and therefore can be a source of disability. In addition, an irregular cartilage bone interface is created and can create an irregular "tidemark" and it is difficult to correctly orient the grafts to regain the exact contour of the femoral condyle.

The indication for AOT is small or medium sized focal full-thickness articular and osteochondral defects of the weight bearing surfaces of the femoral condyles and the patellofemoral joint, which have 1-4cm^2. Large lesions have been treated, but

lesions larger than $8cm^2$ are contraindicated because of the limits on the amount of donor cartilage that can be harvested.

Autologous Chondrocyte Implantation (ACI)

Autologous chondrocyte implantation is a cell-based surgical treatment for large articular cartilage defects. The procedure is performed in two stages. The first stage is an arthroscopic assessment of the cartilage defect and the harvesting of a small of articular cartilage from a relatively non-weight-bearing site within the knee. The harvest sites are usually interchondylar notch or medial margin of trochlea. The cells within this cartilage biopsy specimen are removed from extracellular matrix by enzymatic digestion and then cultured for several weeks until approximately 12 million cells are available for implantation. In the second stage, an open arthrotomy, the repair site is prepared by debridement of the defect, and periosteum harvested from the tibia or femur is sewn over the prepared defect with the cambium layer facing the bone. Fibrin glue is used to seal the margins of the defect. The cultured cells are then injected beneath the periosteal cover. Over a period of weeks to months the cells adhere to the bone and bordering cartilage and provide a hyaline or hyaline-like cartilage.

Allograft osteochondral transplantation

Some full-thickness articular cartilage defects are so large that autologous cartilage procedures do not suffice. In these patients, the defects usually involve the entire femoral condyle or tibial component, i.e. medial or lateral tibial plateau.

Therefore, allograft osteochondral transplantation is a consideration. Gross from Tronto, Canada has the most experience with this technique and is considered a "salvage procedure". The fresh or frozen or fresh osteochondral transplants are harvested en bloc and then securely fixated in place with screw. Unfortunately, the aritcular cartilage does not survive (partially or completely), but clinically can improve the quality of life.

The indications for the type of surgical intervention are a large osteochondral defect involving the majority if not all of the respective compartment that has failed previous less invasive techniques to improve the function and pain.

Evaluation of cartilage repair

Although arthroscopy has been the gold standard for diagnosing and monitoring cartilage damage and repair, it is less than optimal for several reasons. It is invasive and expense. Moreover, this method relies largely on visual inspection of the articular surface for abnormalities such as color changes and fissuring. Arthroscopy also allows probing of cartilage, which involves using a small metallic probe to apply pressure to the cartilage surface to determine if it is softer than normal.

Many imaging methods are available to assess articular cartilage. Conventional radiography can be used to detect gross loss of cartilage evident as narrowing of the distance between the bony components of the joint, but it does not image cartilage directly and is insensitive. Arthography or computed tomographic scanning is also non-invasive and provides information limited to the contour of the cartilage surface.

MRI, with its excellent soft-tissue contrast, is the best technique available for assessment of cartilage injury and repair. Imaging of regions of cartilage damage has the potential to provide morphologic information about the region, such as fissuring, and presence of partial or full-thickness cartilage defect. The many tissue parameters that cane be measured by MR imaging technique have the potential to provide physiological information about repair or transplant as well. The ideal MR imaging technique for cartilage should provide accurate assessment of cartilage thickness, demonstrate morphologic changes of the cartilage surface, demonstrate internal cartilage signal intensity changes, allow evaluation of the subchondral bone signal abnormalities, and predict histology of cartilage.

MRI of articular cartilage

MRI is unique in its ability to allow direct visualisation of the articular cartilage. It is the high water content of articular cartilage that forms the basis for MRI signal on those tissues. This base signal, however, is modulated through a variety of mechanisms by interactions between the tissue water and the collagen and proteoglycan matrix in cartilage. The multiplicity of tissue characteristics that affect image contrast on MRI is the key to this modality's unparalleled capacity for soft-tissue discrimination. The extent to which each of these mechanisms contributes to the overall contrast depends on exactly how the MRI parameters are assigned.

The general goal of articular cartilage imaging is the accurate depiction of the

structure and composition of the tissue to evaluate its integrity. The imaging of articular cartilage proves particularly challenging in this respect because of its zonal changes in structure and biochemical composition over the distance of a few millimetres. The consideration of commonly encountered artefacts associated with MRI adds another dimension of complexity to this imaging dilemma.

Conventional T1- and T2-weighted MR imaging allows depiction of articular cartilage and can demonstrate defects and gross morphologic changes. T1-weighted imaging does not show significant contrast between joint effusion and the cartilage surface, making surface irregularities difficult to detect. T2-weighted imaging capitalises on the arthrogram-like effect produced by the high signal intensity of joint fluid, which accentuates any surface irregularity or focal defects in the articular cartilage.

Normal cartilage on T1-weighted image shows homogenous signal intensity with no multilaminar appearance. As T1-weighted images, cartilage on T2-weighted image homogenous signal intensity in its normal state. Signal alternations generally consist of globular regions of increased signal intensity. The disadvantages of T2-weighted images include decreased spatial resolution as compared with T1-weighted images, a lack of contrast between cartilage and subchondral cortical bone, and insensitivity to the intrinsically short T2 relaxation time of the zones of cartilage.

The accuracy of articular cartilage assessment with MRI has greatly improved

with recent development of imaging sequences designed specifically for hyaline cartilage. The two most widely available techniques use a fat-suppressed three-dimensional spoiled gradient echo sequence or a fast spin-echo sequence. The fast suppressed three-dimensional spoiled gradient-echo sequence visualizes cartilage defects attribute to T1 differences between cartilage and fluid and the fast spin echo sequences visualizes cartilage defects because of T2 differences. Cartilage is higher in signal intensity than fluid on fat-suppressed T1-weighted images and is lower in signal intensity than fluid on intermediate or T2 weighted images.

The fat-suppressed three-dimensional spoiled gradient echo sequence provide high accuracy with overall sensitivity of 86%, specificity of 97%, and accuracy of 91% for detecting of cartilage in the knee. Intermediate or T2-weighted fast spin-echo techniques without or with fat suppression recently have been shown to results in similarly high accuracy with overall sensitivity of 87%, specificity of 94%, and accuracy of 92%. The two imaging techniques have different advantages and disadvantages.

The fat suppressed three-dimensional spoiled gradient echo sequence provides thinner sections, which is advantageous in identifying morphologic defects and for doing three-dimensional analyses. The fast spin-echo sequence is more sensitive for visualization of signal abnormalities within cartilage. The fast spin echo technique is less susceptible to metal artefacts, which can be an advantage when imaging patients

after surgery.

PURPOSE

The purpose of this study is to evaluate quality of the ACI grafts and overall state of the knee based on MR appearances at one year postoperatively, and to assess whether other knee pathologies or features affect the graft quality and to compare these findings with clinical outcome at one postoperative year. This study also was performed to compare the MRI appearances of ACI grafts with graft histology to identify whether MRI can predict the histology.

MATERIALS & METHODS

Ethical Approval

The study was given ethical approval by Shropshire Research and Ethics Committee and all patients were given fully informed consent.

Study Design

The study is a prospective study.

Study Population

Forty-nine patients (35 men, 14 women; age range, 18-51; mean age 35.4 years) were included in the study. All underwent autologous chondrocyte implantation (ACI) of a femoral condyle of the knee. The treatment decision was based on clinical evaluation and arthroscopic appearances in all cases. Only patients with type 3 or 4 femoral condylar cartilage defects, i.e. cartilage thinning or complete defects (Outerbridge classification[1,2]) were treated. Patients who had autologous chondrocyte implantation of a tibia or patella were excluded.

Surgical Procedure

The ACI was performed using the original Gothenberg technique as previously described[3-5]. The procedures were carried out by 2 experienced staff orthopedic surgeons (20 and 30 years experience) who also undertook follow up examinations. Cartilage was arthroscopically harvested from low weight-bearing surfaces of the affected knee. Chondrocytes were enzymatically isolated from the extracellular matrix

with collagenase and cultured in monolayer to increase the cell number. In a second procedure, 3-4 weeks later, the cartilage defect was trimmed back to healthy cartilage and debrided down to subchondral bone, taking care not to injure it. Periosteum was harvested from the proximal tibia and sutured over the defect (6-0 Vicryl). The cultured chondrocytes were injected and the injection site under the periosteal cover sealed with fibrin glue (Tisseal™, Baxter, Vienna, Austria).

MRI Technique and Evaluation

MRI pre-operatively and at one year postoperatively was performed. MRI was performed with a 1.5-T system with 25 mT/m gradient strength (Vision; Siemens, Erlangen, Germany). Patients underwent imaging with a dedicated coil (CP extremity coli; Siemens, Erlangen, Germany).

The following sequences were performed: coronal and sagittal spin-echo T1-weighted (repetition time ms/echo time msec, 722/20; field of view, 20-cm; flip angle, 90°) and gradient-echo T2*-weighted (608/18; field of view, 20-cm; flip angle, 30°) sequences; transverse intermediate-weighted and T2-weighted double-echo sequences (3500/16, 98; field of view, 17.5x20-cm; flip angle; 180°; echo train length, five; spectral fat saturation); and three-dimensional (3D) fat-suppressed fast low-angle shot (FLASH) (50/11; field of view,18-cm; flip angle, 30°; spectral fat saturation) and 3D double-echo steady-state (DESS) (58.6/9; field of view, 18-cm; flip angle, 40°; spectral fat saturation and magnetization transfer) sequences. A standard Siemens

viewing station and software (version VB33A) were used.

MR images were evaluated by two musculoskeletal radiologists (1 and 30 years experience) and one orthopaedic surgeon. After a first independent assessment, all images were reviewed in consensus. If the graft area was not readily identifiable, the surgical notes were consulted. 3D reconstructions were routinely used in the assessment. The radiologists were blinded to the clinical results.

Graft Assessments on MRI

Grafts were evaluated for smoothness and thickness compared to adjacent cartilage, signal intensity, integration to underlying bone and adjacent cartilage and congruity of subchondral bone. Presence of overgrowth of the graft and bone marrow appearance beneath the graft were also assessed.

Graft smoothness was described as irregular or regular. Graft thickness was assessed in comparison with adjacent normal appearing cartilage and graded into three categories of 100% or more (grade III), 50 to 100% (grade II) and less than 50% (grade I). Full thickness defects were in addition recorded separately. The worst part of the graft determined the grade. Signal intensity was graded into 3 groups as similar to normal cartilage, a mixture of similar and low and low or predominantly low intensity compared to that of normal appearing cartilage of the posterior femoral condyle, using a fat suppressed 3D FLASH sequence.

Integration to adjacent cartilage was defined as good where there was continuity

between the graft and the adjacent cartilage, and as poor where a clearly defined high signal intensity cleft was seen between the graft and adjacent cartilage. Integration with bone was defined as poor when a high signal cleft was seen between the graft and underlying bone and as good when continuity of cartilage with the underlying bone was seen. The outline of the subchondral bone was recorded as congruent to the cartilage surface, irregular, a defect, defined as an indentation of the subchondral line into the adjacent marrow, and central osteophyte, defined as a focal protuberance of the subchondral bone into articular cartilage.

Overgrowth of the graft was classified as no overgrowth, partial overgrowth, if only a part of the graft showed overgrowth and general overgrowth if the entire graft demonstrated overgrowth. Bone marrow appearance beneath the grafts was recorded as normal, oedema ("edema-like") if there was decreased signal on T1 and increased signal on T2 or DESS sequences, sclerosis which was low signal on all sequences and cyst formation if there was decreased signal on T1 and increased signal on T2 or DESS sequences with a typical cyst like appearance.

A scoring system for the graft was developed (Table 1). Six features were allocated 0 to 2 points each, resulting in a range of 0 to 12 points. The higher the score, the more advantageous the features. The features making up the graft score were graft smoothness, thickness, signal intensity, integration to adjacent cartilage and underlying bone and congruity of the subchondral bone. This classification was arbitrary and based

on simple assumptions. The grafts were arbitrarily classified into three groups; 11 and more points, between 8 to 10, and 7 and less points. Presence of overgrowth and the bone marrow appearance were evaluated separately (i.e. not included in the scoring system) and in the analysis related to the knee score. A scoring system was used as a proxy to enable multivariant analysis.

Addition to the initial assessment of the graft intensity, the signal intensity was measured on the 3D FLASH images by using the average of 3 measurements at different locations in one image slice deemed representative and free of artifact. The largest possible round regions of interest were used. The signal intensity measurements were compared to the signal intensity of the ipsilateral posterior femoral condylar cartilage and to the average of medial and lateral posterior femoral condylar cartilage.

The thickness of the graft was measured in the 3D FLASH sequence. Generally all sequences were used for the assessment of the graft; only the graft signal intensity measurement was performed solely with the 3D Flash sequence. A magic angle phenomenon was not observed with this sequence.

Assessment of Knee Features on MRI

Presence of osteophytic spurs, cartilage defects, and appearances of the anterior cruciate ligament, the menisci and the subchondral bone beneath the graft were evaluated.

Presence of osteophyte spurs were evaluated in the anterior and posterior edges

of femur and tibia in the sagittal plane, the superior and inferior edges of the patella in the sagittal plane and the medial and lateral edges of the tibiofemoral joint compartments in the coronal plane. The osteophytes were not graded by size but the number of osteophytes was used for classification.

Presence and location of cartilage defects additional to the graft sites were recorded. The appearance of the anterior cruciate ligaments were described as intact, partial tear, tear or reconstruction. Menisci were described as intact, degenerative, partially removed or tear.

A scoring system for these features was developed (Table 2). The higher the score is, the more disadvantageous the appearance is. Each feature was attributed 0 to 2 points, the maximum score was 8 points. The scores were grouped into three classes; score 0: normal, score 1 to 3: moderate pathology, and score of more than 4: severe pathology. All sequences were used for the assessment of knee features.

Clinical Evaluation

A patient-assessment questionnaire modified of Lysholm score was used for clinical outcome[6]. This score uses subjective (pain etc.) and objective (range of movement etc.) criteria, the higher the score the better the knee, the maximal score is 100. Forty-eight patients in 49 completed the questionnaire preoperatively and postoperatively. The postoperative score was not available for one patient.

Tissue Biopsy and Histological Evaluation

Twelve months after the ACI procedure the patient underwent arthroscopy of the affected knee after MRI examination. During arthroscopy a perpendicular full thickness biopsy of the center of the graft was taken using a 1.8mm diameter bone marrow biopsy needle (Manatech, Stoke-on-Trent, UK). A mapping system was used to ensure the correct location. The biopsy was taken from the center of the graft. The cores were taken as near to 90° to the articulating surface as possible. Cores were snap-frozen in liquid nitrogen-cooled hexane and stored in liquid nitrogen. Frozen sections 7 μm thick were collected onto poly-L-lysine coated slides with haematoxylin and eosin.

The biopsy was assessed by a cartilage research scientist (member of the ICSR Histology Endpoint committee) using polarized and plain light microscopy for the collagen organization and morphology.

The tissue was categorized into 4 morphological groups according to its appearance when viewed with polarized light as recommended by the International Cartilage Repair Society[7,8]. 'Hyaline cartilage' described tissue where the extracellular matrix had a truly glass-like appearance and cells were oval, often in a lacuna typical of chondrocytes (Figure 1). 'Fibrocartilage' described tissue where bundles of collagen fibers were obvious and random within the cartilage matrix with elongated cells resembling fibroblasts (Figure 2). 'Mixed' tissue describes a combination of hyaline and

fibrocartilage. 'Fibrous' tissue was diagnosed, when blood vessels were obvious and the tissue was looser and more disorganized (Figure 3).

Statistical Analysis

Wilcoxon t-test was used for comparison between preoperative and one-year postoperative clinical scores. Kruskal-Wallis H-test was used for comparisons between the groups, and Mann-Whitney U-test with Bonferroni method was performed as post-hoc test.

Interobserver variation was assessed by calculating r-values using the Pearson correlation and kappa scores for parametric and non-parametric data. Analysis of variance was performed for the assessment of the relation of graft signal intensity to graft histology. Chi square values were determined for the graft histologies versus loss of graft thickness, graft overgrowth, graft surface smoothness, graft integration to adjacent cartilage and underlying bone, bone marrow changes underneath the graft and bone marrow contours underneath the graft.

Statistical assessment was performed using a standard statistical software package, SPSS v12. Statistical significance was defined as $P<0.05$.

RESULTS

Details of individuals and their graft score, knee features score, histology and clinical score was shown on Table 3.

Graft Appearances on MRI

All grafts could be evaluated. Susceptibility artefact was seen in the grafts in all cases, however, it did not prevent adequate assessment of the graft. The results of graft assessment are shown in Table 4.

Thirty-two grafts of 49 (65%) had 100% or more thickness compared to adjacent cartilage (Figure 4). 5 grafts (11%) had more than 50% thickness and 6 grafts (12%) had less than 50% and 6 grafts (12%) demonstrated thickness loss reaching to bone (Figure 5). General overgrowth was seen in 8 grafts (16%) (Figure 6), and partial overgrowth in 13 grafts (26%) (Figure 7). The graft surface was irregular in 32 grafts (65%) (Figure 8). The signal intensity of the graft was high or predominantly high in 33 grafts (67%) and predominantly low or low in 7 grafts (14%), and a mixed pattern was seen in 9 grafts (19%).

Integration to the adjacent cartilage was good in 42 grafts (86%) and poor in 7 grafts (14%). Integration to the underlying bone was good in 47 grafts (96%) and poor in 2 grafts (4%). The subchondral bone was classed as congruent in 23 (47%), central osteophyte was seen in 1 (2%), bone surface defects in 10 grafts (20%) and bone surface irregularity in 15 grafts (31%). Six knees with osteochondral defects preoperatively

demonstrated a persistent bone defect (Figure 2).

1 year postoperatively the bone marrow beneath the grafts appeared normal in 18 knees (37%) and abnormal in 31 knees (63%). Oedema beneath the graft was observed in 27 grafts (47%) (2 patients had oedema and sclerosis and four patients had oedema and subcortical cysts), subcortical cyst formation in 6 grafts (12%) (4 of these had cysts with oedema) and sclerosis in 4 grafts (8%) (2 of these had sclerosis with oedema) (Figure 4,9).

Both preoperative and postoperative MR examinations were available for 41 patients. In 11 knees (27%) bone marrow oedema had developed underneath the graft site compared with the pre-op defect site. In 4 knees (10%) cyst formation developed underneath the graft compared with the pre-op examination. 7 cases had bone marrow oedema deep to the defect site on the preoperative examination and in four cases persistence of oedema was seen underneath the graft at 1 year follow up, while in 3 cases the oedema had disappeared. In one case cyst formation was demonstrated underneath the defect site preoperatively and this cyst had disappeared on 1 year follow up.

Knee Features on MRI

At 1 year follow up there was no osteophyte formation in 21 (43%) of cases. 15 knees (31%) had one to four osteophytes and 13 knees (26%) had more than five osteophytes. Of 41 knees where preoperative MR examinations were available, four

knees. which had no osteophyte preoperatively had one to two osteophytes at one year postoperatively.

Thirty-eight (77%) knees had an intact ACL. 8 knees (16%) had an ACL reconstruction before or at the time of the ACI procedure. In 3 knees (7%) a tear of the ACL was seen on preoperative and one-year follow up MR examination. 32 additional cartilage defects in 23 knees (48%) were observed one year postoperatively; 20 in the femoral condyles, 8 in the patella and 4 in the tibia. Fourteen knees (28%) had intact menisci, and 26 knees had degenerative or partially removed menisci. Nine knees had meniscal tears on MR imaging.There was no change of note compared to the pre-operative MRIs.

Clinical Outcome

The mean self-assessment Lysholm score was 50.7 preoperatively and rose to 72.3 points one-year postoperatively (P=0.000003).

Histology

Biopsy was taken from forty-one patients. Histological examination revealed hyaline cartilage in 4 patients. mixed fibrohyaline cartilage in 10. fibrocartilage in 25 patients and fibrous tissue in 2 patients.

Correlations

Mean graft score was 8.7 (95% CI; 8.0 to 9.5). The mean score was 0.7 points for graft smoothness. 1.4 for graft thickness, 1.5 for signal intensity, 1.7 for integration

to adjacent cartilage, 1.9 for integration to underlying bone, and 1.5 for the congruity of subchondral bone. 15 grafts (31%) had more than 11 points, 20 grafts had 10 to 8 points and 14 grafts (28%) had less than 7 points.

Relating the classification of osteophytes with the general graft score, grade one patients (no osteophytes) had a significantly higher graft score than grade three patients (>11 osteophytes) (P=0.0057) (Table 5). There is also a significant relation of the graft score with cartilage defects, between grade one (no additional defect) and grade three (>1 additional defect) knees (P=0.014) (Table 6). There was no significant relationships of the graft score with the ACL status (Table 7), the menisci (Table 8) and graft overgrowth (Table 9). There was also no relationship between the graft score and underlying bone marrow changes (Table 10). There was no correlation between the graft score and the clinical outcome scores.

For overall knee features, normal knees with score 0 had a mean graft score of 10.0 points (95% CI; 8.8 to 11.2). Knees with moderate pathology had a mean graft score of 9.0 (95% CI; 8.2 to 9.8), and knees with severe pathology had a mean graft score of 7.5 (95% CI; 5.7 to 9.3) (Figure 12).

Knees with 11 or more points for the graft score had a mean 23.8 points rise of clinical score (95% CI; 12.8 to 34.8). Knees with a graft score between 8 and 10 had a mean 24.7 points rise (95% CI; 13.3 to 20.7), and knees with 7 or less points had 14.9 points rise in clinical score (95% CI; 1.5 to 28.3). (Figure 13). There was no statistically

significant correlation between the severity of overall knee features and the graft score at one year (p=0.2644), and there was no statistically significant correlation between the graft score at 1 year and the rise in the clinical knee score (P=0.3834). However there was a significant relation of the absolute Lysholm score with the graft score (P=0.014). "better" grafts are associated with higher clinical scores (Figure 13).

There was no correlation of graft histology and graft thickness (P=0.83) (Table 11). Similarly the histological composition was recorded versus graft overgrowth. There was no relationship between the overgrowth of the graft and its histological type (P=0.69) (Table 12). Overgrowth was seen in altogether 16 grafts (39.0%). 10 of these were partial and 6 general overgrowth. Overgrowth occurred in areas of strong and light weight bearing. A cartilage defect opposite the overgrown graft was not seen. There was no relationship between the graft histology and the graft surface smoothness (P=0.28) (Table 13). The integration of the graft to adjacent cartilage and the underlying bone (Table 14) was compared with the graft histology groups; poor integration to underlying bone was present only once when an ill defined high signal area was seen between the graft and the bone on the 3D sequences. All biopsies demonstrated good integration of the graft tissue to the underlying bone. There was no statistical correlation of graft integration to adjacent cartilage and underlying bone to the graft histology (P=0.90).

The appearance of the bone marrow was compared for the different graft histologies. There were 23 (56%) grafts with edema-like signal of the underlying bone

marrow (Table 15). Four of these showed additional cyst formation. Normal appearances of the bone marrow underneath the graft were seen in 18 cases. No statistically relevant correlation of bone marrow status and graft histology was found (P=0.63). The bony contour underneath the graft (Table 16) demonstrated no statistically relevant difference between the different histological groups (P=0.94). Central osteophytes underneath the graft were seen in 1 case only.

The signal intensities of the grafts were compared to that of the cartilage of the ipsilateral posterior condyle and the average of both posterior condyles; the ratio of the signal intensity of the graft versus the posterior condyle signal intensity was plotted against the graft histology and resulted in virtually identical figures (Figure 14). The signal intensity ratio did not differ for different types of graft histology (P=0.34). The signal intensity of the graft was almost always lower than that of adjacent normal cartilage.

The interobserver correlation of the signal intensity measurements of the grafts was poor (r=0.37) while the average signal intensity for all grafts were almost identical for the two observers. Kappa values for the observations of graft smoothness (κ=0.21), graft overgrowth (κ=0.53) and graft thickness (κ=0.41) were calculated.

DISCUSSION

ACI procedures have been introduced for the treatment of cartilage lesions of weight bearing joints. Follow up routines vary and may center on clinical symptoms or include direct visualization via arthroscopy or indirect via MRI[7,9-15]. There are no trials establishing a follow up protocol and while experience with MR imaging exists it often has been performed on symptomatic patients with limited follow up of larger patient collectives.

This study represents one of the first prospective studies evaluating MR appearance of ACI in the knee, histology and clinical outcome, and to the best of our knowledge is the first to evaluate its correlations with other knee features. This study shows that graft overgrowth does not seem to have great if any significance. The significance of continued presence of bone marrow oedema remains unclear. Multiple osteophytes and further cartilage defects result in worse ACI grafts at 1 year follow up. The rise of the clinical score is not significantly related to a better clinical score but the absolute clinical score is. This study also shows that MRI can not predict ACI graft histology.

The cartilage and ACI graft was assessed using 3D sequences which provide superior spatial resolution and the combination of the FLASH and the DESS sequences provides excellent contrast resolution. The FLASH sequence has been acknowledged as having major value in the evaluation of cartilage[16,17] but the DESS sequence in addition

provides a clear evaluation of the outline of the cartilage surface which is not always clearly seen on the FLASH sequence. In the experience of the authors, assessment of the cartilage or graft can be difficult with only one of these sequences. The FLASH sequence has also been verified for volumetric measurements and has been recommended for cartilage evaluation[18,19]. Susceptibility artefacts of the graft were present in some cases but they did not prove to be a significant problem and the assessment of the graft was always possible. The sequences used in this study differ to some extent with those recommended by the International Cartilage Repair Society (ICRS) which have been published since commencement of the study[15]. The sequences used in this study are more comprehensive as they include an additional 3D study and T1 weighted studies in place of the intermediate weighted (proton density) sequences. It has been important to preserve the imaging protocol to ensure comparability over time for this cohort study. The main disadvantage of the imaging protocol used is its long acquisition time.

Limitations in sensitivity of FLASH and DESS sequences for cartilage abnormalities and flaring are well recognized and opinions vary regarding which sequence is superior for cartilage imaging [20-23].All patients in this study were examined with at least two 3D sequences (and several 2D sequences) and this increased confidence in differentiating cartilage from joint effusions. Window settings needed to be adjusted carefully during review of a case. Irregularities of the graft surface on MRI

have been described previously and seem to be relatively common [10-14]. They were also frequently seen on arthroscopy [24].

The classification of knee and graft features was created for this study and has not been validated. Classifications were based on a common sense approach based on the question of which role other degenerative features in the knee joint play in relation to the success of the graft and to the clinical outcome.

In this study, complete filling of defects by the grafts were seen in 32 grafts of 49 (65%) and there was some graft overgrowth in 42% of cases. This is similar to the reported 40% incidence of overgrowth in patients undergoing repeat arthroscopy of whom 7 (27%) had symptoms (15). This overgrowth has been attributed to overgrowth of periosteum and in one series, 11 of 50 patients had recurrent symptoms of pain, swelling or catching of whom six had hypertrophy of the periosteal patch[25]. Micheli et al[26] recorded that 25% of a series of patients had symptoms requiring a second look arthroscopy, the majority of which (80%) had periosteal hypertrophy. The results of our study using MR on all patients regardless of symptoms suggest that some overgrowth is frequently present at the one year stage and may be asymptomatic. This is considerably higher than the 12% reported by Henderson et al[10] who also used MR for evaluation. We have no explanation for this difference. Our study has found that there was no difference between the clinical outcome scores for the partial or complete overgrowth or the absence of overgrowth and this was also the situation in the study by Henderson et

al[10]. The reason for graft overgrowth is not clear but the assumption that this is periosteal hypertrophy is not supported by our histological studies on biopsies of our patients at one year which did not identify the periosteum as the cause of overgrowth in the specimens[12].

The graft signal intensity has been reported to vary between patients and to reflect the maturity of the graft[12]. Immature grafts may have relatively higher signal compared to normal cartilage with 83% showing some areas of hyperintensity at three months using T1. T2 and proton density sequences[10]. At one year 63% were identical and 30% slightly hyperintense to adjacent cartilage[10]. Using fat suppressed FSE sequences immature grafts have also been reported to have slightly higher signal intensity to normal cartilage whereas mature intact grafts may have similar intensity to normal cartilage, mildly higher or lower, often heterogenous with a layered or speckled pattern, which were present at about 6 months post procedure. In the present study all graft signal evaluations were made on 3D- flash fat-suppressed images and at one year 67% of the grafts were of similar intensity to the articular cartilage of the adjacent posterior femoral condyle with some heterogeneity. Overall in our patients the average graft signal has been lower than posterior femoral condylar cartilage and hyperintensity to normal cartilage has not been a feature regardless of the graft appearances on histology. Delayed gadolinium-enhancement MR imaging suggest that the glycosaminoglycan index in ACI grafts 6 months or less postoperatively is about 59%

and at 12 to 24 months increased to about 91% of controls and therefore the graft may not reach maturity until one year or later[27]. In this study there is no clear correlation between the signal intensity of the graft and clinical outcome which was also the findings of Henderson et al[10].

Abnormal appearances were present in the bone marrow deep to the graft in 63% of patients at one year compared to 27% of the 41 patients who had pre-procedure MR studies. Subchondral cysts were also present in six cases with four having associated bone oedema. Bone marrow oedema was also present in 62% of subjects at three months in the report of Henderson et al[10] but had reduced to 41% by one year with the majority of these being classified as mild. Alparslan et al[12] reported bone marrow oedema-like signal deep to the graft is a normal finding in the early postoperative period but that in mature grafts the marrow signal is usually normal or has minimal linear bright signal on fat suppressed images. No data is provided for this observation. Recht et al[15] describe the presence of oedema like signal but indicate that the length of time that it persists has yet to be determined. This study indicates that marrow signal changes are common and may be present prior to the ACI procedure. It is possible that the incidence of bone marrow oedema in this study is underestimated as the T2 and DESS sequences are less sensitive for demonstrating oedema than a STIR sequence which was not included in this study. The reason for the higher proportion of cases with persistent bone marrow oedema is unclear but the high percentage of cases with pre-procedure

oedema may be an influence. No information is provided by other authors of the preprocedure incidence of bone marrow oedema but it is interesting to note that only three of our patients had resolution of preprocedure changes at one year.

The pathological cause for the bone marrow oedema pattern has been examined by Zanetti et al[28] in osteoarthritic knees using STIR sequence and correlating the findings with histology. These authors found that the majority of areas that were abnormal on MRI were normal on microscopy with 53% fatty marrow, 16% intact trabeculae and 2% blood vessels. Abnormal findings on histology included bone marrow necrosis in 11%, abnormal trabeculae in 8%, bone marrow fibrosis in 4%, bone marrow edema in 4% and bone marrow bleeding in 2%.

The morphological correlate and the clinical significance of bone marrow oedema after ACI remains unclear. Our study has found no correlation with the clinical score or the subchondral bone changes at one year, but it is possible that persistent oedema may be more significant and longer term follow up of patients will be required to make this evaluation.

The quality of the knees undergoing grafting has been analysed in this study. Knees without osteophytes or additional cartilage defects had a significantly higher graft score than the remaining knees (table4,5). One year postoperatively the rise in clinical score was lower in the group with multiple osteophytes but not to a statistically significant level. However the absolute knee score had a significant relation to the graft

quality. This suggests that even though the association of poorer grafts with overall worse knee was not statistically significant in this study, this association is likely to be present.

The relationship between graft quality, osteophyte formation and multiple cartilage defects may reflect altered contact pressure of the femoral and tibial condyles. Excessive contact pressure has been shown to occur at sites of joint space narrowing and the contact area has been shown to increase in relation to the grade of osteoarthritis[29]. Areas of degenerative lesions have been shown to have highly non-uniform contact pressures and it is suggested that high contact pressures may lead to cartilage breakdown[24,30]. There is no published data on contact pressure on ACI grafts at one year in the patients with differing degrees of degeneration but altered contact pressures may result in a poorer quality of graft.

Knees with no or little degenerative cartilage lesions, ACL abnormalities or osteophyte formation had higher graft scores and higher improvement of clinical scores at one year than knees without evidence of significant degenerative change. However apart from the graft scores in relation to osteophyte formation and cartilage defects these findings were not statistically significant to a $p<0.05$ level.

The findings in this study are consistent with those of Minas et al[9] who reported that knees with isolated unipolar lesions preoperatively had a superior clinical outcome at two to three years after surgery compared to knees with radiographic or

arthroscopic evidence of osteoarthritis. However, unlike our study, the clinical score preoperatively in these two groups were similar. Minas[25] combined osteotomy in many of his complex and salvage patients and therefore the final results in these groups are difficult to compare with this series. Two to 9-year outcome by Peterson et al[31] reported that knees with multiple lesions or with ACL reconstruction had inferior clinical outcome to those with isolated femoral defects or osteochondritis dissecans.

The number of patients assessed in the study is limited and the small numbers and wide range of clinical responses has resulted in some comparisons not reaching statistical significance which otherwise might have done so. Longer term follow-up will be of interest to clarify a number of questions raised by this study in particular the relevance of high signal intensity of the graft and changes to the bone marrow underlying the graft.

There is a general assumption that ACI grafts forming hyaline like cartilage do better than other grafts. To assess the histological composition arthroscopy is necessary which is invasive and can only assess the joint surface. MR imaging offers non invasive assessment of all joint structures but can not assess the histology. This study tries to correlate the histological outcome with MR imaging features.

Most grafts studied were composed of a mixture of fibrohyaline- or fibrocartilage (35/41). Hyaline cartilage was less common (4/41) and fibrous tissue was rare (2/41). In all cases at least part of the graft had survived at 1 year follow up. The histological

assessment was performed according to the criteria of the International Cartilage Repair Society [7,8,32] which requires the use of ordinary light and polarized light microscopy. Not utilizing polarization in the assessment process is known to result in higher proportions of hyaline like cartilage [7,8,24,32].

The further comparison of MR features of the ACI grafts with the graft histology was based on this assessment.

The relative thickness of the graft 1 year after implantation was not dependent on graft histology. This needs to be confirmed in longer term follow up but could indicate that graft survival and possibly function is not dependent on the histological type.

Graft overgrowth is attributed to overgrowth of the periosteal patch [12,13,26,31,33] with histological studies demonstrating a top layer of fibrous tissue which is suggested to originate from periosteum [24]. In this study there was no obvious relationship of overgrowth and histology of the grafts. The relevance of graft overgrowth is not yet determined.

A trilaminar or layered appearance of the graft as suggested by some authors was not seen in this study.

No relationship between the histological composition of the graft and the graft signal intensity or intensity ratio could be demonstrated. Previous smaller studies cited differences in signal intensity between graft and adjacent cartilage as a feature of poor

prognosis. These studies centered on clinically symptomatic patients though [12-14]. In this study there has been no statistically relevant relation between graft histology and the MR features examined and therefore this study does not seem to confirm this. This study seems to indicate that on 1 year follow up persistence of decreased signal intensity in the graft is not abnormal. Edema-like signal underneath the graft was not correlated to any particular histology of the graft.

The classification and scoring system of graft features and other knee features have not been validated but there is no established and proven scoring system and the choice of criteria and classifications represents a common sense approach to identify outcome factors in this study.

Limitations

The imaging protocol for this study does not represent the current recommendations [15] but reflects the practice at the beginning of the study. The authors of the study presented here believe that the sequences are adequate for graft and joint assessment. The 3D sequences (DESS and FLASH) complement each other in their characteristics. Further the 3D FLASH sequence is to the authors' knowledge still the only sequence validated for volume measurements [19,34,35].

The interobserver correlation for graft signal intensity was poor which probably reflects the free choice of images for signal intensity measurements. Kappa scores for the various MR features of the graft were also only modest.

CONCLUSIONS

ACI grafts on MR imaging at one year follow up have a spectrum of appearances. Bone marrow oedema deep to the graft and graft overgrowth are common and do not appear to have an adverse prognostic significance. Grafts with advantageous features such as good integration to adjacent cartilage and underlying bone and no significant thickness defects are more frequently found in otherwise well preserved knees. The presence of osteophytes and other cartilage defects results in poorer ACI grafts at 1 year. Advantageous graft and knee features are associated with a better absolute clinical outcome but not with the relative improvement. In other words, better grafts are associated with clinically better knees but even for suboptimal grafts there is improvement. However, MRI can't predict ACI graft histology; histology was not related to graft signal intensity, thickness, overgrowth, surface smoothness, integration to adjacent cartilage or underlying bone, signal change in underlying bone marrow.

MR imaging of ACI grafts allows for serial follow up of patients non-invasively. It can assess the entire graft and its integration to adjacent bone and cartilage. It allows assessment of the entire remainder of the knee. With development of higher field scanners and progress in sequence development it can be expected that resolution and contrast will increase while imaging time decreases.

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Table 1
Graft score

Classification	grade I	grade II	grade III
Score	**0**	**1**	**2**
Smoothness	Irregular	-	Regular
Thickness	< 50%	> 50%, < 100%	>= 100%
Intensity	Predominantly low or low	Mixture	Predominantly high or high
Integration to cartilage	Poor	-	Good
Integration to bone	Poor	-	Good
Congruity of subchondral bone	Central osteophyte or Defect	Irregular	Congruent

Various ACI graft features are assigned scores from 0 to 2: the grading is arbitrary

Table 2
Overall knee feature score

	Classification	grade I	grade II	grade III
	Score	**0**	**1**	**2**
Osteophyte spur (number)		0	1 to 4	5 to 13
Cartilage defect (number)		0	1	more than 1
ACL		Intact	Partial tear or Reconstruction	Tear
Menisci		Intact	Partial menisectomy or Degenerative	Tear

Various knee joint features are assigned scores from 0 to 2; the grading is arbitrary

Table 3
Details of individuals and their graft score, knee features score and histology

Patients	Sex and Age	Location of defect	Size of defect (mm)	Interval* to MRI/Biopsy (month)	Graft score (0~12)	Knee feature score (0~6)	Histology type	Lysholm score at one year postopratively (0~100)
1	27/M	MFC	26x23	12/17	12	4	Fibro/Hyaline	99
2	36/F	trochlea	14x30	12/10	9	3	Fibrocartilage	89
3	41/M	LFC	10x10	13/13	6	5	Fibrocartilage	41
4	29/M	MFC	30x20	12/12	7	3	Fibro/Hyaline	63
5	26/M	LFC	25x20	12/16	9	4	Fibrocartilage	52
6	37/F	MFC	25x12	11/14	9	1	Fibrocartilage	25
7	32/M	MFC	18x20	12/12	10	4	Fibro/Hyaline	78
8	28/F	MFC	27x17	12/12	11	0	Fibro/Hyaline	100
9	54/F	MFC	30x20	12/13	9	0	Fibrocartilage	99
10	22/M	MFC	17x14	12/13	12	3	Fibrocartilage	78
11	35/F	MFC	25x30	12/12	8	1	Fibrocartilage	26
12	38/M	MFC	20x18	11/12	8	2	Fibrocartilage	83
13	28/M	MFC	32x17	12/10	7	2	Fibrocartilage	84
14	33/M	MFC	50x15	13/19	9	2	Fibrocartilage	72
15	39/M	MFC	20x20	11/11	10	4	Fibrocartilage	59
16	26/M	LFC	27x21	12/19	11	1	Fibrocartilage	91
17	39/M	MFC	30x20	12/12	9	6	Fibrocartilage	98
18	45/M	MFC	25x8	12/13	10	3	Fibro/Hyaline	99
19	34/M	MFC	14x8	12/12	12	0	Fibro/Hyaline	80
20	44/F	MFC	10x10	11/-	12	1	-	21
21	41//M	MFC	35x32	12/13	7	5	Fibro/Hyaline	77
22	25/M	LFC	16x13	12/12	12	1	Fibrous tissue	84
23	42/M	MFC	25x13	12/12	9	0	Fibrocartilage	13
24	39/M	MFC	-	12/12	4	6	Fibrocartilage	79
25	43/F	MFC	18x15	12/12	11	2	Fibrocartilage	86
26	38/M	LFC	28x13	11/15	9	2	Hyaline	84

Table 3 (continued)
Details of individuals and their graft score, knee features score and histology

Patients	Sex and Age	Location of defect	Size of defect (mm)	Interval * Biopsy/MRI (month)	Graft score (0~12)	Knee feature Score (0~6)	Histology Type**	Lysholm score at one year postopratively (0~100)
27	32/M	LFC	24x25	11/12	12	2	Fibro/Hyaline	52
28	49/M	MFC	25x10	11/12	4	3	Fibrocartilage	55
29	31//M	MFC	30x20	11/-	11	1	-	73
30	48//F	LFC	20x20	11/12	8	1	Hyaline	87
31	19/F	MFC	10x15	12/13	9	2	Fibrocartilage	42
32	34/M	MFC	38x25	11/12	7	3	Fibro/Hyaline	40
33	42/F	LFC	23x22	11/12	12	4	Fibrous tissue	73
34	30/F	LFC	20x17	18/-	9	3	-	95
35	29/M	LFC	24x22	12/12	2	5	Fibrocartilge	91
36	30/M	MFC	31x23	11/-	4	4	-	36
37	32/M	LFC	20x20	11/12	12	7	Fibrocartilage	80
38	33/M	MFC	10x10	12/12	10	4	Fibrocartilage	97
39	36/M	MFC	25x20	12/-	11	2	-	87
40	36/M	Trochlea	16x18	10/-	8	0	-	94
41	21/M	MFC	20x30	11/12	11	1	Fibrocartilage	100
42	29/F	MFC	27x15	11/12	11	4	Fibrocartilage	74
43	43/M	MFC	30x30	11/15	4	3	Fibrocartilage	94
44	39/M	MFC	15x25	12/8	7	2	Fibro/Hyaline	13
45	41/M	MFC	30x14	12/12	11	0	Fibrocartilage	45
46	33/F	LFC	29x19	10/10	6	3	Hyaline	95
47	44/M	MFC	60x22	12/-	2	6	-	78
48	52/M	MFC	20x30	11/-	9	2	-	82
49	32/F	MFC	25x15	12/-	10	2	-	100

* interval between ACI procedure and biopsy or MRI
* Hyaline: hyaline cartilage. Fibro/Hyaline: mixed fibrohyaline cartilage

Table 4
Result of graft assessment 1 year post ACI

Smoothness	Regular: 17	Irregular: 32		
Thickness	>100%: 32	50 to 100%: 5	0< 50%: 6	Reach to bone: 6
Intensity	High*: 33	Mixture: 9	Low*: 7	
Integration to cartilage	Good: 42	Poor: 7		
Integration to bone	Good: 47	Poor: 2		
Congruity of subchondral bone	Congruent: 23	Irregular: 15	Defect: 10	Central osteophyte: 1
Overgrowth of the graft	No: 28	Partial: 13	General: 8	
Bone marrow beneath the graft	Normal: 18	Oedema: 27	Sclerosis: 4**	Cyst: 6***

* including predominantly high or low
** including 2 cases with sclerosis and oedema
*** including 4 cases with cyst formation and oedema

Table 5

Osteophyte spur classification versus associated graft score and rise of clinical knee score

Grade	No of osteophytes	Number	Graft score	Rise of clinical score
I	0	20	9.7* (8.8 to 10.5)	22.7 (10.2 to 35.1)
II	1 to 4	16	9.0 (7.8 to 10.2)	24.0 (10.8 to 36.0)
III	5 to 13	13	6.9 (5.2 to 8.6)	17.9 (4.1 to 31.8)

( ): 95% confidence intervals
* class I vs III; significant difference (P=0.0057)

Table 6

Cartilage defect classification versus associated graft score and rise of clinical knee score

Grade	No of additional cartilage defects	Number	Graft score	Rise of clinical score
I	0	25	9.5* (8.7 to 10.3)	21.5 (10.9 to 32.1)
II	1	18	8.4 (7.1 to 9.8)	27.1 (14.4 to 39.7)
III	>1	6	6.3 (3.9 to 8.7)	6.0 (-4.8 to 16.8)

( ); 95% confidence intervals
* class I vs III; significant difference (P=0.014)

Table 7

ACL classification versus associated graft score and rise of clinical knee score

Grade	ACL	Number	Graft score	Rise of clinical score
I	Intact	38	8.8 (8.1 to 9.6)	22.7 (13.6 to 31.7)
II	Partial tear or Reconstruction	8	8.9 (6.2 to 11.5)	21.0 (6.9 to 35.1)
III	Tear	3	6.7 (3.8 to 9.5)	10.0 (3.7 to 16.3)

( ): 95% confidence intervals: no statistically significant relation

Table 8

Menisci classification versus associated graft score and rise of clinical knee score

Grade	Menisci	Number	Graft score	Rise of clinical score
I	Intact	19	9.3 (8.4 to 10.2)	18.0 (5.2 to 30.7)
II	Meniscectomy or Degeneration	22	8.7 (7.5 to 9.9)	23.5 (13.2 to 33.8)
III	Tear	8	7.4 (5.2 to 9.6)	25.3 (5.7 to 44.8)

( ); 95% confidence intervals; no statistically significant relation

Table 9
ACI graft overgrowth versus graft score and rise of clinical knee score

	Number	Graft score	Rise of clinical score
No*	28	8.1 (7.1 to 9.2)	19.9 (9.5 to 30.3)
Partial	13	9.5 (8.2 to 10.7)	24.5 (10.9 to 38.2)
General	8	9.6 (8.6 to 10.7)	23.0 (5.8 to 40.2)

( ); 95% confidence intervals; no statistically significant relation
* including cases with loss of thickness

Table 10

Bone marrow appearance beneath the graft versus graft score and rise of clinical knee score

	Number	Graft score	Rise of clinical score
Normal	18	9.4 (8.4 to 10.4)	26.8 (12.0 to 41.6)
Oedema, sclerosis	25	8.5 (7.4 to 9.6)	16.5 (7.8 to 25.2)
Cyst	6*	7.7 (5.2 to 10.1)	27.3 (20.1 to 34.6)

( ): 95% confidence intervals: no statistically significant relation
* including 2 cases with oedema

Table 11

Loss of thickness of the ACI graft for the different histology groups (relative thickness loss compared to adjacent normal cartilage)

	No thickness loss	Thickness loss, <50%	Thickness loss, >50%	Full thickness defect
Hyaline cartilage	2	1	1	0
Mixed	8	0	1	1
Fibrocartilage	14	4	3	4
Fibrous tissue	2	0	0	0

no statistically relevant correlation, P=0.83

Table 12
ACI graft overgrowth (compared to adjacent normal cartilage) for the different histology groups

	No graft overgrowth	Partial overgrowth	General overgrowth
Hyaline cartilage	3	1	0
Mixed	5	4	1
Fibrocartilage	16	4	5
Fibrous tissue	1	1	0

no statistically relevant correlation, P=0.69

Table 13

ACI graft histology surface smoothness (as seen on the 3D cartilage sequences) for the different histology groups

	Smooth graft surface	Irregular graft surface
Hyaline cartilage	1	3
Mixed	4	6
Fibrocartilage	8	17
Fibrous tissue	2	0

No statistically relevant correlation, P=0.28

Table 14
ACI graft integration to adjacent cartilage and underlying bone for the different histology groups

	Adequate integration to adjacent cartilage	Poor integration to adjacent cartilage	Adequate integration to underlying bone	Poor integration to underlying bonet
Hyaline cartilage	4	0	4	0
Mixed	10	0	10	0
Fibrocartilage	20	5	24	1
Fibrous tissue	2	0	2	0

No statistically relevant correlation. P=0.90

Table 15

ACI graft bone marrow changes underneath the graft for the different histology groups (in all patients with cyst formation bone marrow edema was also present

Bone Marrow underneath graft	Normal	Oedema	Cyst formation
Hyaline cartilage	2	2	0
Mixed	4	6	0
Fibrocartilage	12	13	4
Fibrous tissue	0	2	0

No statistically relevant correlation, P=0.63

Table 16
ACI graft bone contour underneath the graft for the different histology groups

Bone outline Underneath graft	Congruent	Central osteophyte	Defect	Irregularity
Hyaline cartilage	2	0	1	1
Mixed	6	0	1	3
Fibrocartilage	10	1	5	9
Fibrous tissue	2	0	0	0

No statistically relevant correlation, P=0.94

Figure 1
Histology of "Hyaline cartilage"

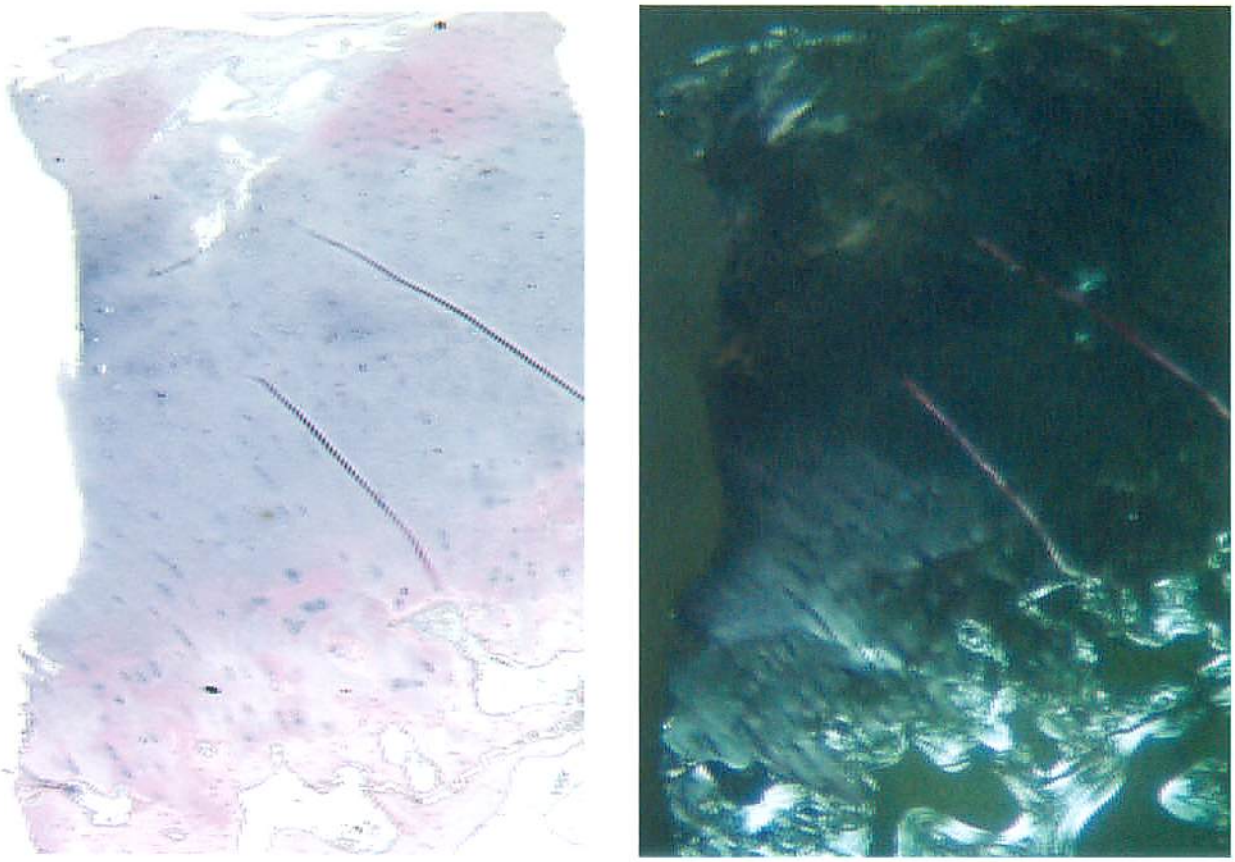


Figure 2
Histology of "Fibrocartilage"

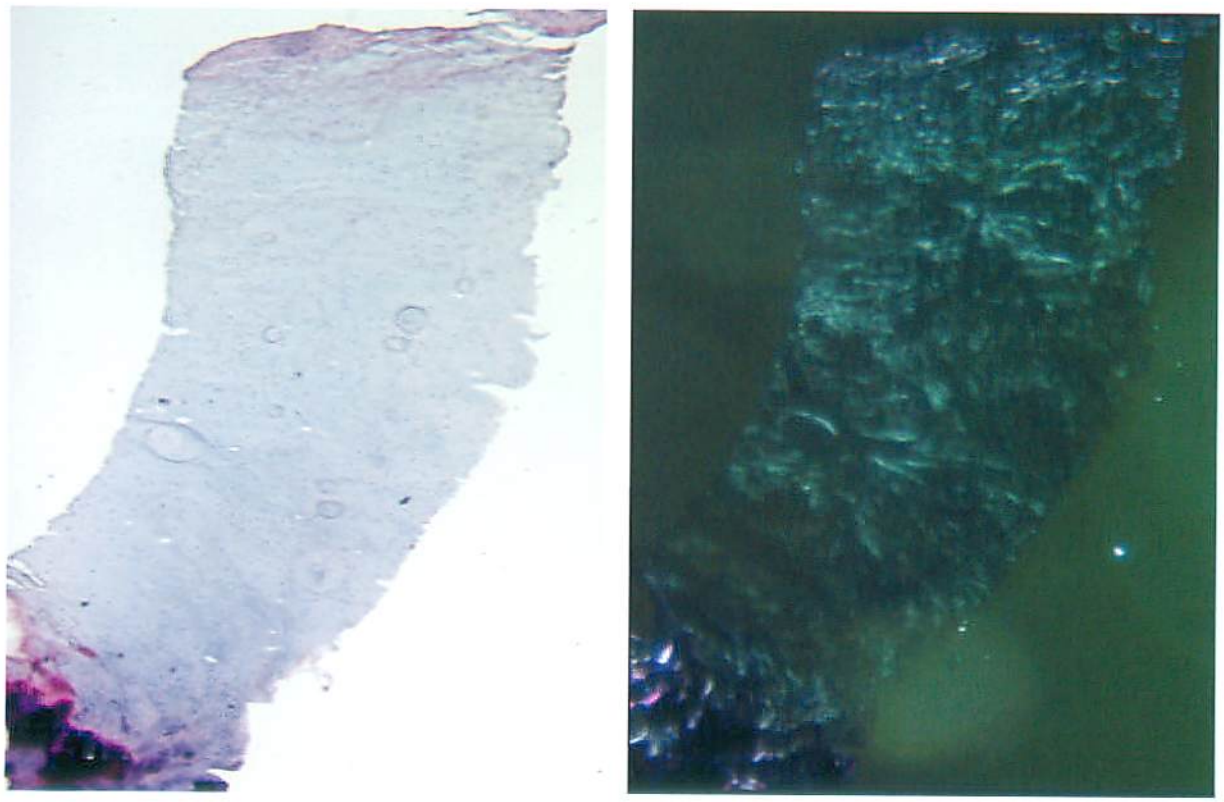
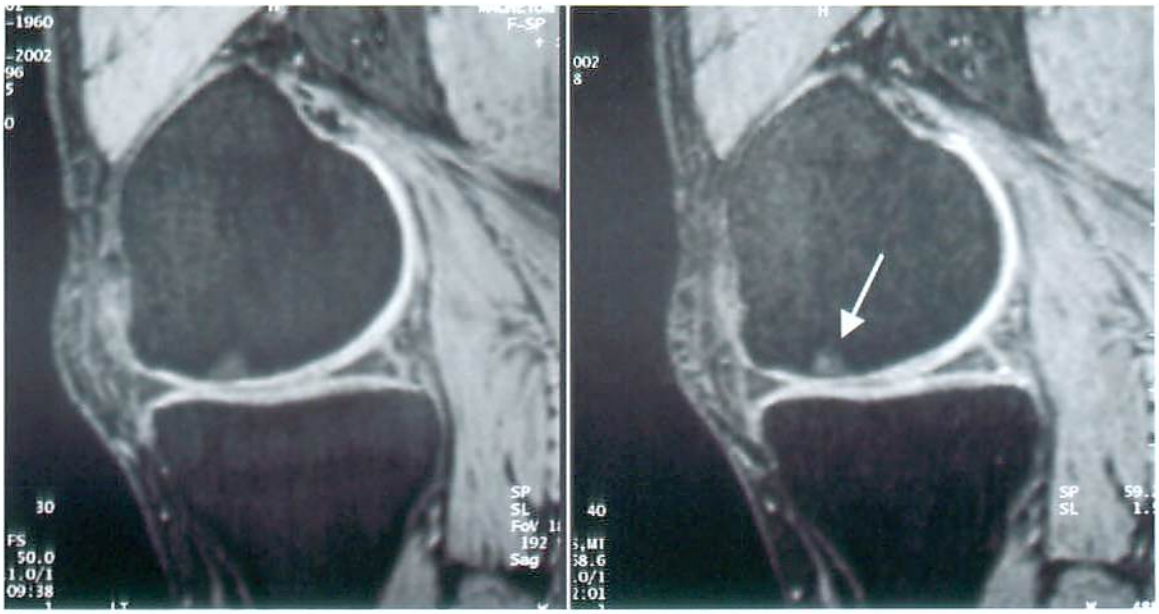


Figure 3
Histology of "Fibrous tissue "

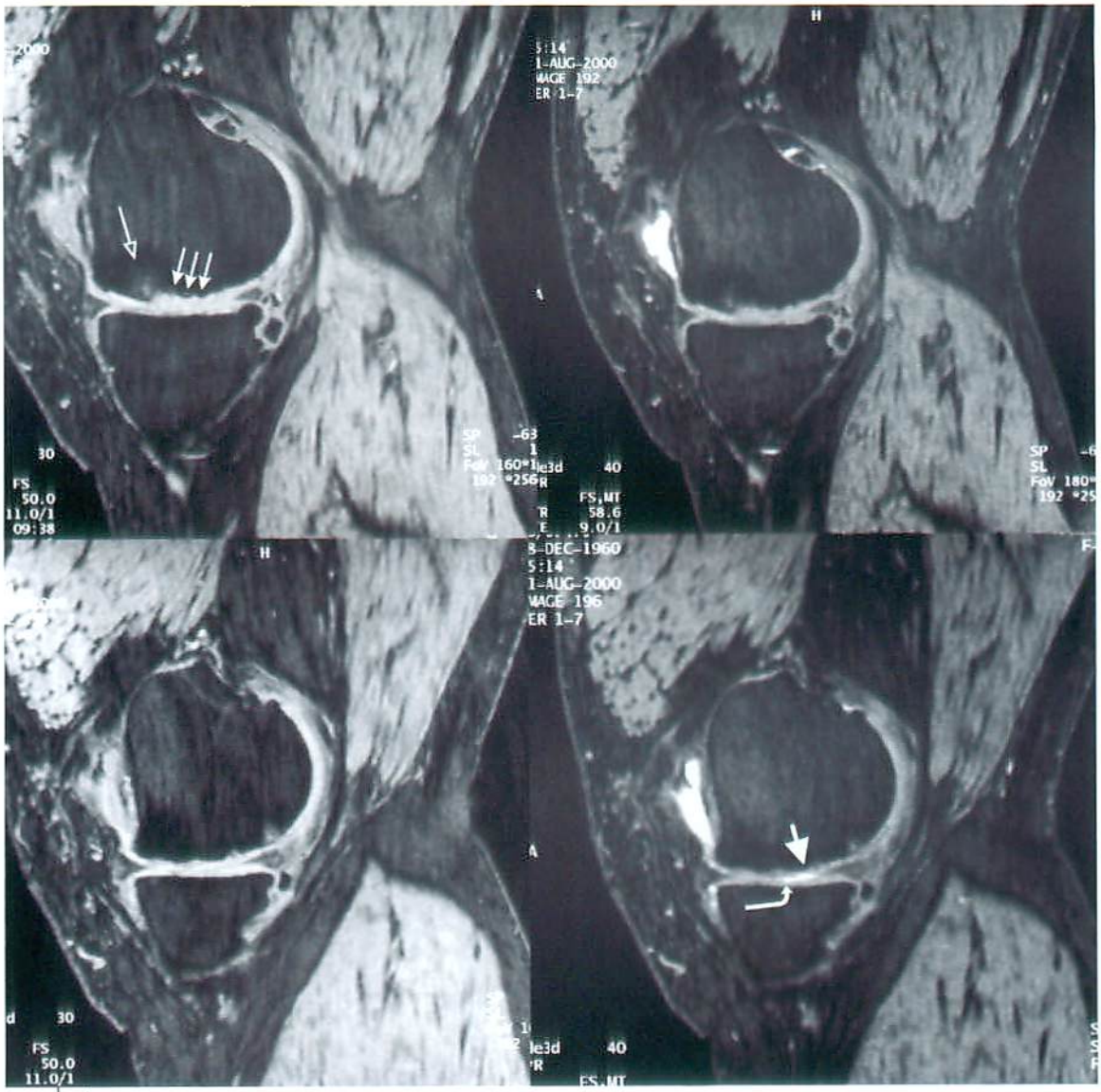
Figure 4
42 year old male patient; ACI 1 year follow up



a) b)

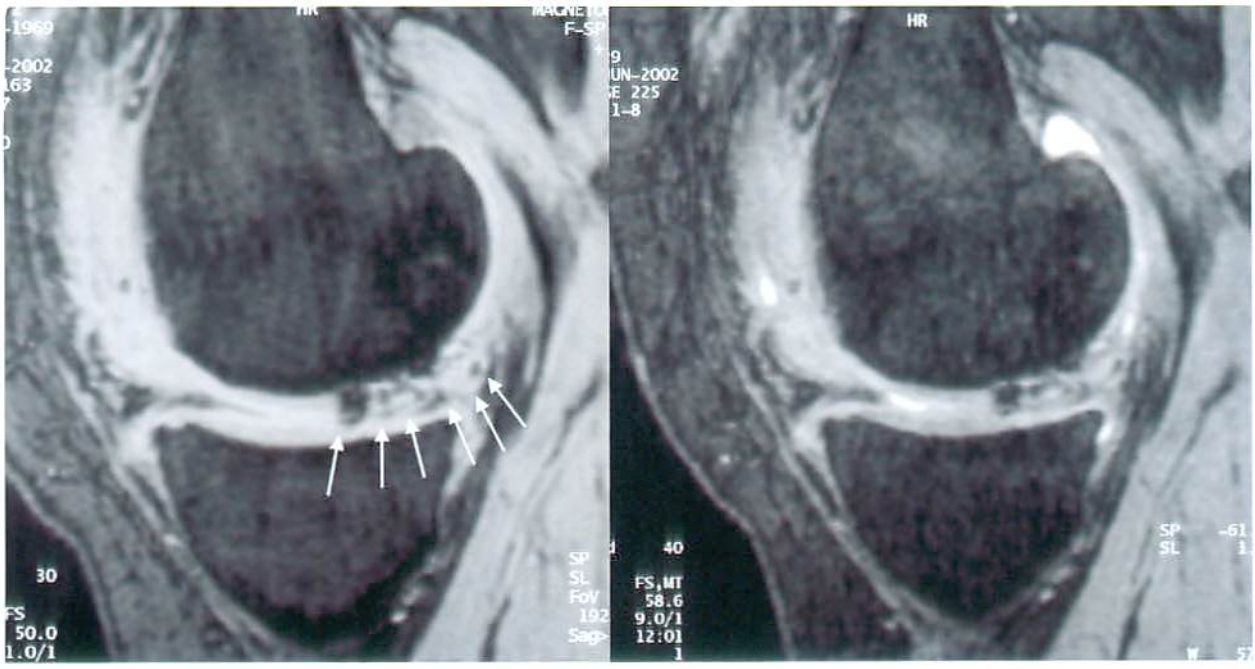
Sagittal FLASH (TR/TE 50/11, 18-cm field of view, 30° flip angle, spectral fat saturation) (a) and DESS (TR/TE 58.6/9, 18-cm field of view, 40° flip angle, spectral fat saturation and magnetization transfer) (b). The graft shows virtually no artifact and is not distinguishable from adjacent normal cartilage. Underlying bone marrow oedema is however noted (arrow).

Figure 5
40 year old male patient; ACI 1 year follow up



Sagittal FLASH (TR/TE 50/11, 18-cm field of view, 30° flip angle, spectral fat saturation) (a,c) and DESS (TR/TE 58.6/9, 18-cm field of view, 40° flip angle, spectral fat saturation and magnetization transfer) (b,d). Underneath the large graft irregular bone contour (arrows) is seen with oedema of the adjacent bone marrow (open arrow) (a,b). The graft contour is smooth. Further lateral (b,d) the graft has a defect (thick arrow) and there is cartilage thinning in the opposite tibial plateau (curved arrow). Further posteriorly bone marrow oedema in the femoral condyle. Osteophyte formation is also noted.

Figure 6
33 year old female patient; ACI 1 year follow up



a) b)

Sagittal FLASH (TR/TE 50/11, 18-cm field of view, 30° flip angle, spectral fat saturation) (a) and DESS (TR/TE 58.6/9, 18-cm field of view, 40° flip angle, spectral fat saturation and magnetization transfer) (b). Generalized overgrowth of the graft (arrows), and marked artifact is seen.

Figure 7
45 year old female patient; ACI 1 year follow up

a) b)

Sagittal FLASH (TR/TE 50/11, 18-cm field of view, 30° flip angle, spectral fat saturation) (a) and DESS (TR/TE 58.6/9, 18-cm field of view, 40° flip angle, spectral fat saturation and magnetization transfer) (b). Partial overgrowth of the graft (arrows), which is otherwise well integrated. Some artifact is seen.

Figure 8
40 year old male patient; ACI 1 year follow up

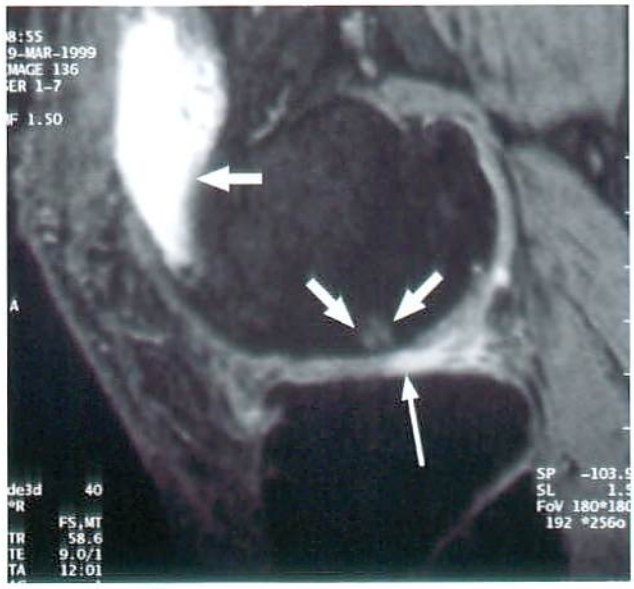
a)

b)

Sagittal FLASH (TR/TE 50/11, 18-cm field of view, 30° flip angle, spectral fat saturation) (a) and DESS (TR/TE 58.6/9, 18-cm field of view, 40° flip angle, spectral fat saturation and magnetization transfer) (b). There is little artifact (short arrow) and mildly decreased signal intensity of the graft (long arrows) compared to adjacent cartilage. The graft is well integrated, with a similar thickness as the adjacent cartilage; smooth graft surface.

Figure 9
43 year old male patient; ACI 1 year follow up

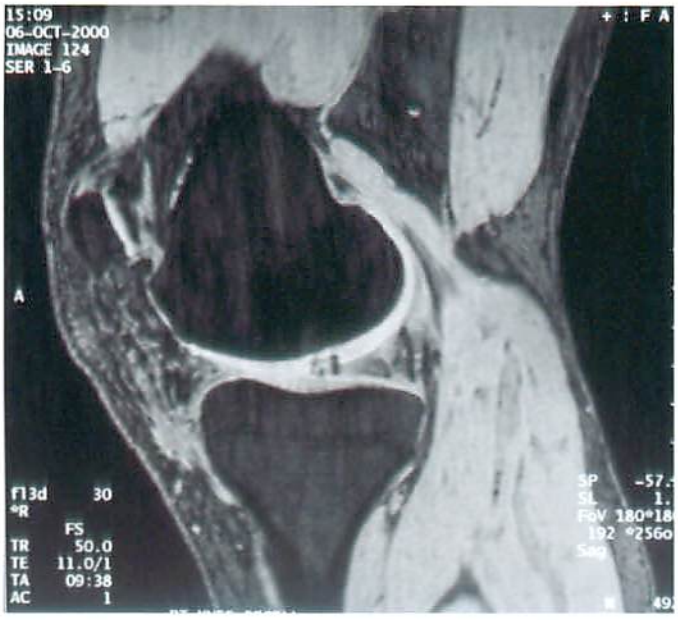
a)



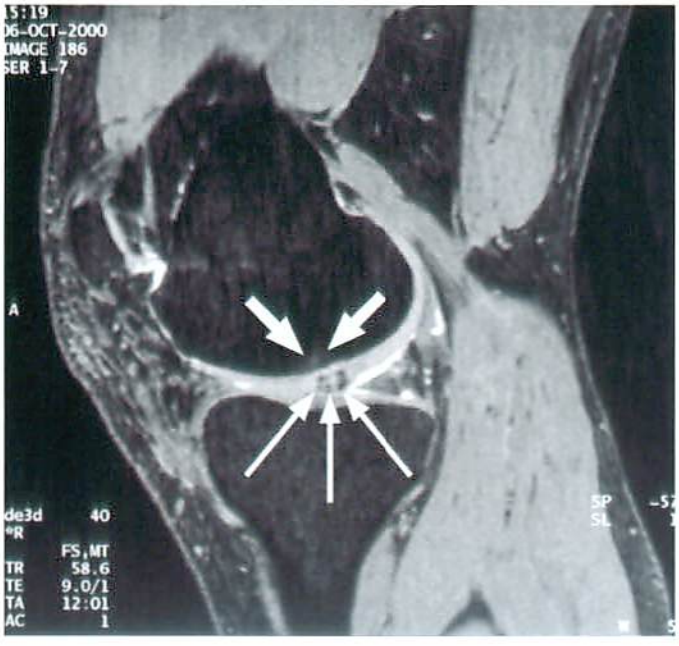
b)

Sagittal FLASH (TR/TE 50/11, 18-cm field of view, 30° flip angle, spectral fat saturation) (a) and DESS (TR/TE 58.6/9, 18-cm field of view, 40° flip angle, spectral fat saturation and magnetization transfer) (b). There is a full thickness defect in the graft seen (long arrow) with edema like signal of underlying bone marrow (short arrows) and marked narrowing of tibial cartilage opposite. The features are much better appreciated on the DESS sequence. Osteophytes and severe degeneration of the lateral meniscus. Joint effusion (horizontal arrow).

Figure 10
33 year old male patient; ACI 1 year follow up



a)



b)

FLASH sagittal FLASH (TR/TE 50/11, 18-cm field of view, 30° flip angle, spectral fat saturation) (a) and DESS (TR/TE 58.6/9, 18-cm field of view, 40° flip angle, spectral fat saturation and magnetization transfer) (b). Overgrowth of the graft (long arrows), which is otherwise well integrated. Some artifact is seen. Note also the subtle bone marrow edema underneath the graft (short arrows).

Figure 11
45 year old male patient; ACI 1 year follow up

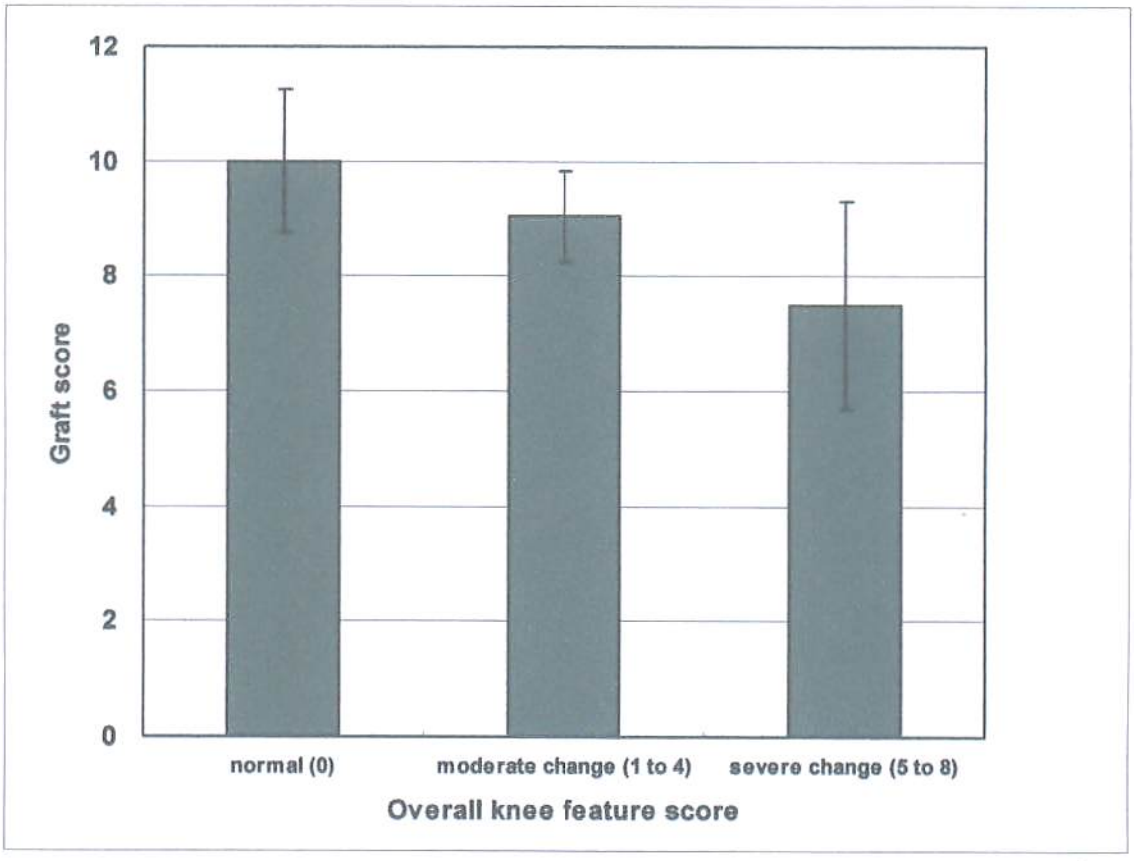
a)

b)

Sagittal FLASH (TR/TE 50/11, 18-cm field of view, 30° flip angle, spectral fat saturation) (a) and DESS (TR/TE 58.6/9, 18-cm field of view, 40° flip angle, spectral fat saturation and magnetization transfer) (b). Generalized loss of thickness of the graft (arrows) with an irregular surface and decreased signal intensity compared to normal adjacent cartilage. Oedema like signal is present in bone marrow in the posterior femoral condyle (horizontal arrow) unrelated to the graft site. Osteophytes and subchondral cysts are present in the anterior tibia.

Figure 12

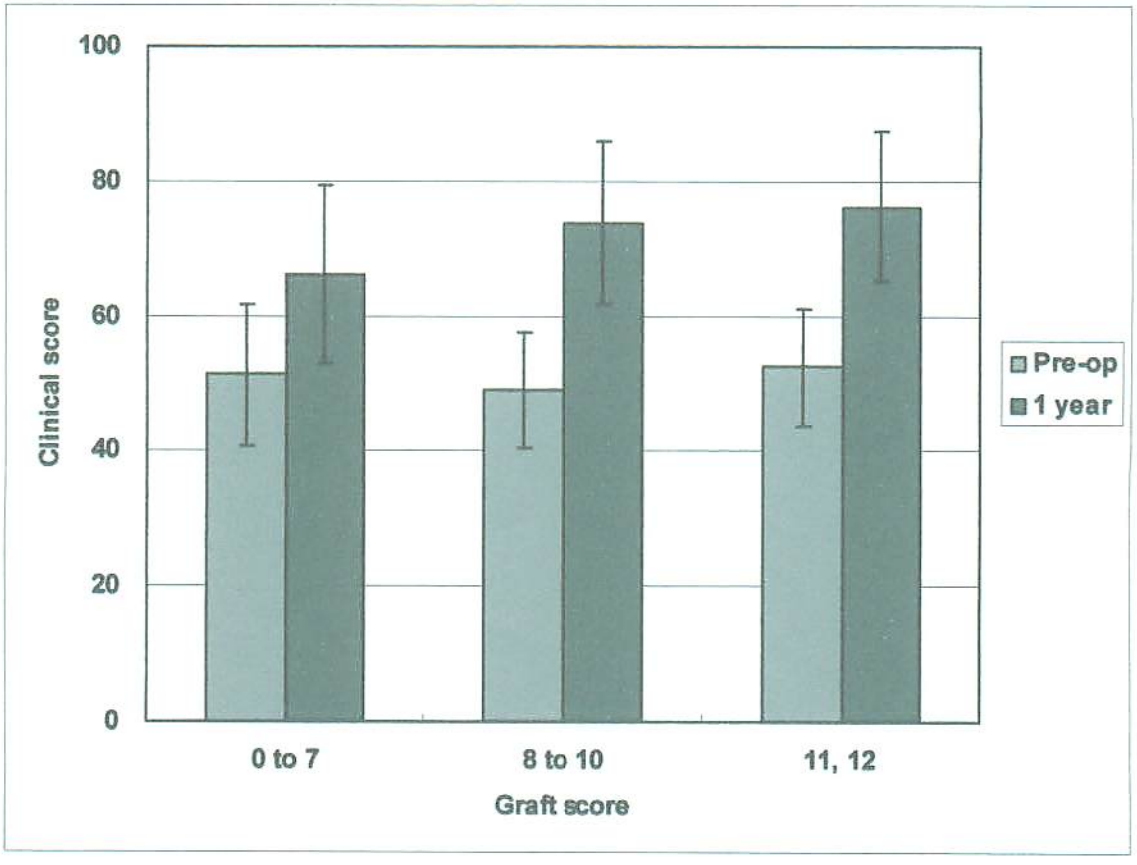
Comparison of the graft score (higher score "better" graft) with knee feature score (higher score "worse" knee)



Differences are not statistically significant. However when assessing the presence of *osteophytes* or further cartilage defects separately, these are associated with worse graft outcomes.

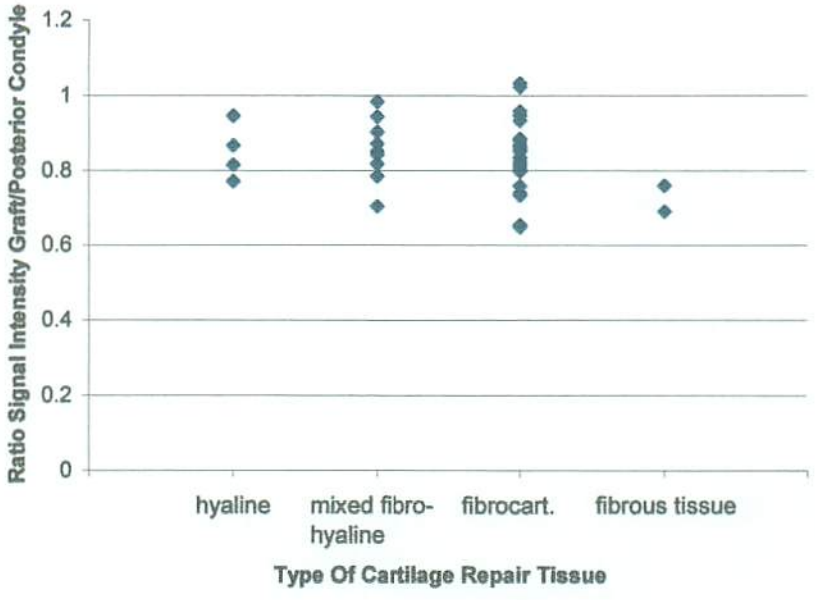
Figure 13

Comparison of the clinical (Lysholm-) score (higher score equals better clinical status) with the graft score (higher score "better" graft)



There is no statistically relevant relation of the rise of the Lysholm score versus the graft score but there is a statistically relevant relation (P=0.014) of the absolute clinical score 1y post ACI of graft score 0-7 vs. absolute clinical score of graft score 8-10

Figure 14
Ratio of signal intensity of graft and average of the posterior femoral condyles



Measured in 3D FLASH sequence; (virtually identical result for the ipsilateral cartilage only); no statistically relevant correlation of graft histology and signal intensity (P=0.34), prediction of graft histology is not possible by measuring signal intensity